

Page 33, line 8, cancel "succinimide" and insert
--succinamide--.

Please cancel the claims and insert the following new claims:

(a) incubating a sample of the biological fluid with (i) a ligand analog tracer which, due to its chemical structure, does not bind to some of the endogenous binding proteins, (ii) a specific ligand binder at a concentration which does not significantly strip bound ligand from said endogenous proteins and having an affinity constant from about 0.246×10^5 up to about 5×10^5 l/mol and, (iii) at least one specific chemical inhibitor reagent that inhibits the binding of the ligand analog tracer to other endogenous binding proteins, said specific chemical inhibitor reagent being present in a concentration sufficient to displace the ligand analog tracer from at least one other endogenous binding protein without

(b) separating the ligand analog tracer bound to the specific binder from unbound tracer; and

(c) determining the concentration of free ligand in said biological fluid.

Claim 36. A method for measuring the concentration of free thyroxine or triiodothyronine free ligands in biological fluids in the presence of bound ligand and endogenous binding proteins, including albumin, without disturbing the equilibrium between the free ligand and the protein bound ligand, comprised of the following steps:

(a) incubating a sample of biological fluid with (i) a ligand analog tracer which, due to its chemical structure, does not bind to some of the endogenous binding proteins, (ii) a specific ligand binder at a concentration which does not significantly strip bound ligand from said endogenous proteins and having an affinity constant from about 0.246×10^5 up to about 5×10^5 l/mol and, (iii) specific chemical inhibitor reagents that alone or in combination inhibit the binding of the ligand analog tracer to other endogenous binding proteins, said specific chemical inhibitor reagents being present in a concentration sufficient to displace the ligand analog tracer from at least one other endogenous binding protein without

displacing the native ligand from said endogenous binding proteins;

(b) separating the ligand analog tracer bound to the specific binder from unbound tracer; and

(c) comparing the bound fraction in said sample to the bound fraction of a given set of known free ligand calibrators to determine the concentration of free ligand in said biological fluid.

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Claim 37. The method of claim ²⁸ 35 wherein the chemical inhibitor agent is 2,4-dinitrophenol at a concentration of 5-10 mmol/l.

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Claim 38. The method of claim ²⁸ 35 wherein the chemical inhibitor agent is sodium salicylate at a concentration of 40-125 mmol/l.

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Claim 39. The method of claim ²⁸ 35 wherein the chemical inhibitor reagent is sulfobromophthalein at a concentration of 0.8×10^{-5} M to 1.6×10^{-5} M.

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Claim 40. The method of claim ²⁸ 35 wherein the chemical inhibitor reagent is oleic acid at a concentration of 0.4-0.8 mmol/l.

Claim ³⁴~~41~~. The method according to claim ²⁸35 or ²⁹36 wherein the specific ligand binder is an antibody to said free ligand.

Claim ³⁵~~42~~. The method according to claim ²⁸35 or ²⁹36 wherein the specific ligand binder is immobilized on a solid substrate.

Claim ³⁶~~43~~. A method according to claim ³⁵42 wherein the solid substrate is polypropylene.

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CONT.
Claim ³⁷~~44~~. The method according to claims ²⁸35 to ²⁹36 wherein the ligand analog tracer is labelled with at least one radioactive atom, an enzyme, fluorophor, light chromophore or chemiluminescent group.

Claim ³⁸~~45~~. The method according to claim ³⁷44 wherein the ligand analog tracer is N-¹²⁵I-L-triiodothyronine succinimide or N-¹²⁵I-L-thyroxine succinimide.

Claim ³⁹~~46~~. The method according to claims ²⁸35 or ²⁹36 when carried out at about 37°C and at about pH 7.4.

Claim ⁴⁰~~48~~. The method according to claim ²⁹36 wherein said free ligand calibrators have been prepared by adding different amounts of the ligand to ligand-free human serum, calibrating by equilibrium dialysis and assigning free ligand values.

Respectfully submitted,

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